

***Achnanthidium tinea* sp. nov. – a new monoraphid diatom (Bacillariophyceae) species, described on the basis of molecular and morphological approaches**

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Academic editor: K. Manoylov | Received 3 November 2020 | Accepted 21 December 2020 | Published 12 March 2021

Citation: Tseplik ND, Maltsev YI, Glushchenko AM, Kuznetsova IV, Genkal SI, Kociolek JP, Kulikovskiy MS (2021) *Achnanthidium tinea* sp. nov. – a new monoraphid diatom (Bacillariophyceae) species, described on the basis of molecular and morphological approaches. PhytoKeys 174: 147–163. <https://doi.org/10.3897/phytokeys.174.60337>

Abstract

A new monoraphid diatom species *Achnanthidium tinea* Tseplik, Kulikovskiy, Kociolek & Maltsev, **sp. nov.** is described from Indonesia. The species is described on the basis of molecular and morphological analyses. According to molecular data the new species belongs to the clade that includes strains of *Achnanthidium minutissimum*, *Achnanthidium saprophilum* and *Achnanthidium digitatum*. Morphologically, the new species differs quite significantly from other species of the same genus because of linear-elliptic valves with almost parallel sides and strongly radiate striae and a butterfly-shaped fascia on the raphe valve. The morphology and phylogeny of the new species are discussed, and thoughts on the current state of the taxonomy of the genus *Achnanthidium* are expressed. Our work shows the importance of using molecular data in diatom systematics and also demonstrates the need to investigate rarely studied regions of our planet.

Keywords

Achnanthidium, Bacillariophyceae, Indonesia, molecular investigations, new species

Introduction

The genus *Achnantheidium* Kützing was first described by Kützing (1844) and for a long time it was considered a subgenus of *Achnanthes* Bory s.l. (Cleve 1895). Its status as a separate genus was restored by Round et al. (1990) and afterwards Round and Bukhtiyarova (1996) proposed a new diagnosis which significantly narrowed the genus boundaries. The improved diagnosis included such features as small linear-lanceolate to elliptic-lanceolate valves, radiate uniseriate striae, external distal raphe ends that are straight or curved to one side and sternum that widens in the center of the valve. Currently, two morphological groups are distinguished within the genus: the *Achnantheidium minutissimum* (Kützing) Czarnecki species complex has straight external distal raphe ends, while the *Achnantheidium pyrenaicum* (Hustedt) Kobayashi species complex has external distal raphe ends that are distinctly curved in one direction (Kobayashi 1997). A third group, previously recognized for *A. exiguum* (Grunow) Czarnecki and its relatives (Karthick et al. 2017), has been established as a separate genus, *Gogorevia* Kulikovskiy, Glushchenko, Maltsev & Kociolek (Kulikovskiy et al. 2020c).

Recent studies include descriptions of many new species belonging to this genus (Rimet et al. 2010; Kulikovskiy et al. 2011; Novais et al. 2011; Krahn et al. 2018; Yu et al. 2019, etc.), as well as studies of type materials of known species using light and scanning electron microscopy (Hlúbíková et al. 2011; Van de Vijver et al. 2011). The studies of type materials primarily concern large species complexes and their main aim is to define separate species more clearly.

Taxonomy within the genus *Achnantheidium* is a rather complicated issue. Species boundaries are often not clear enough due to the fact that morphological features alone may not be sufficient to unequivocally identify species, and because values of quantitative features often overlap in similar species, further complicating their separation (Kulikovskiy et al. 2016a; 2020b; Jahn et al. 2017; Tseplik et al. 2020). These problems require extensive molecular research, both while describing new species and while studying species already known to science. At present, the *Achnantheidium* genus includes about two hundred species (Kociolek et al. 2020b). Molecular data is available for very few taxa, and correct identification of the representatives of this genus based only on morphological features is often difficult (Kulikovskiy et al. 2014, 2016a; Andreeva et al. 2016; Maltsev and Kulikovskiy 2017; Maltsev et al. 2018, 2019).

Achnantheidium species are widely distributed in various freshwater habitats around the world and can be important indicators of environmental conditions (Ponder and Potapova 2007). However, many regions remain poorly studied and the probability of finding new species is quite high, like in Lake Baikal (Kulikovskiy et al. 2011, 2012, 2013, 2015, 2016b, c, 2020b) or Southeast Asia (Kulikovskiy et al. 2018; Liu et al. 2018; Glushchenko et al. 2016, 2017, 2018, 2019, 2020; Kezlya et al. 2020). Hustedt (1937a–c, 1938a, b; 1939; 1942) first documented freshwater diatoms from Indonesia, and of the nearly 800 taxa reported, 315 of them (ca. over 40%) were new to science. Still, Indonesia is a country not only with a high level of

endemism in many groups of living organisms, but also taxa still to be discovered or reinterpreted (Hamsher et al. 2014; Kapustin et al. 2017, 2019, 2020; Kociolek et al. 2018; Kulikovskiy et al. 2019b, 2020a; Rybak et al. 2019). Of the 39 monoraphid diatoms reported in the genera *Cocconeis* Ehrenberg and *Achnanthes* Bory by Hustedt (1937a–c, 1938a, b; 1939) alone, 10 (26%) were described as new. There have been no modern taxonomic studies of *Achnanthidium* in Indonesia. The purpose of the present report is to provide light and scanning electron microscopic observations, as well as DNA sequence data, in support of the description of a new *Achnanthidium* species from Sulawesi, Indonesia.

Materials and methods

Sample collection

The sample used in the present report was collected from Indonesia by I.I. Ivanov on 22.09.2010, and designated I227 from the Sulawesi Island, Temple Lake, periphyton, scraping from macrophytes, $t=26.5\text{ }^{\circ}\text{C}$, $\text{pH}=8.7$, conductivity= $277\text{ }\mu\text{S cm}^{-1}$, $04^{\circ}06.923'\text{N}$, $119^{\circ}58.613'\text{E}$.

Culturing

Monoclonal strains were established by micropipetting single cells under an inverted microscope. Non-axenic unialgal cultures were maintained in WS liquid medium (Andersen 2005) for one month. The strain investigated here was designated Ind296.

Preparation of slides and microscopic observation

The sample and the monoclonal culture were treated with 10% hydrochloric acid to remove carbonates and washed several times with deionized water for 12 hours. Afterwards, the samples were boiled in concentrated hydrogen peroxide ($\approx 37\%$) to dissolve organic matter. After decanting and refilling up to 100 ml with deionized water, the suspension was spread on to coverslips and left to dry at room temperature. Permanent diatom preparations were mounted in Naphrax (refraction index = 1.73). Light microscopic (LM) observations were performed with a Zeiss Axio Scope A1 microscope equipped with an oil immersion objective ($\times 100$, n.a. 1.4, differential interference contrast) and AxioCam Erc 5s camera (Zeiss). Valve ultrastructure was examined using a JSM-6510LV scanning electron microscope (IBIW, Institute for Biology of Inland Waters RAS, Borok, Russia).

For scanning electron microscopy (SEM), parts of the suspensions were fixed on aluminum stubs after air-drying. The stubs were sputter-coated with 50 nm Au in an Eiko IB 3. Sample and slides are deposited in the collection of MHA, Main Botanical Garden Russian Academy of Science, Moscow, Russia. The type slide was designated 04133.

All images acquired from the slides were processed using Adobe Photoshop CC (19.0). Length and breadth of the valves were measured on the LM images, and striae and areolae density was measured on the SEM images. The numbers given in brackets in the description are means with standard deviations.

Molecular investigations

Total DNA of monoclonal cultures was extracted using InstaGene Matrix according to the manufacturer's protocol. A fragment of 18S rDNA (382 bp, including V4 domain) was amplified using primers D512for and D978rev following Zimmermann et al. (2011). Amplification of the 18S rDNA fragment was carried out using the pre-made mix ScreenMix (Evrogen, Russia) for the polymerase chain reaction (PCR). The conditions of amplification for 18S rDNA fragment were: an initial denaturation of 5 min at 95 °C, followed by 35 cycles at 94 °C for denaturation (30 s), 52 °C for annealing (30 s) and 72 °C for extension (50 s), and a final extension of 10 min at 72 °C.

The resulting amplicons were visualized by horizontal agarose gel electrophoresis (1.5%), colored with SYBR Safe (Life Technologies, United States). Purification of DNA fragments was performed with the ExoSAP-IT kit (Affimetrix, USA) according to the manufacturer's protocol. 18S rDNA fragment was decoded from two sides using forward and reverse PCR primers and the Big Dye system (Applied Biosystems, USA), followed by electrophoresis using a Genetic Analyzer 3500 sequencer (Applied Biosystems).

Editing and assembling of the consensus sequences were carried out by comparing the direct and reverse chromatograms using the Ridom TraceEdit program (ver. 1.1.0) and Mega7 (Kumar et al. 2016). Newly determined sequence and DNA fragments from 151 other diatoms, which were downloaded from GenBank (taxa and Accession Numbers are given in the Suppl. material 1), were included in the alignments. Three centric diatom species were chosen as the outgroups. The nucleotide sequences of the 18S rDNA gene were aligned separately using the Mafft v7 software and the E-INS-i model (Kato and Toh 2010). The resulting alignment had lengths of 404 characters.

The dataset was analyzed using the Bayesian inference (BI) method implemented in Beast ver. 1.10.1. (Drummond and Rambaut 2007) to construct phylogeny. For each of the alignment partitions, the most appropriate substitution model was estimated using the Bayesian information criterion (BIC) as implemented in jModelTest 2.1.10 (Darriba et al. 2012). This BIC-based model selection procedure selected TIM1+I+G model, shape parameter $\alpha = 0.4210$ and a proportion of invariable sites (pinvar) = 0.3400. We used the GTR model of nucleotide substitution instead of TIM1, given that it was the best matching model available for the Bayesian inference method. A Yule process tree prior was used as a speciation model. The analysis ran for 15 million generations with chain sampling every 1000 generations. The parameters-estimated convergence, effective sample size (ESS) and burn-in period were checked using the software Tracer ver. 1.7.1. (Drummond and Rambaut 2007). The initial 25% of the trees were removed, the rest retained to reconstruct a final phylogeny. The phylogenetic tree and posterior probabilities of its

branching were obtained on the basis of the remaining trees, having stable estimates of the parameter models of nucleotide substitutions and likelihood. Maximum Likelihood (ML) analysis was performed using the program RAxML (Stamatakis et al. 2008). The nonparametric bootstrap analysis with 1000 replicates was used. The statistical support values were visualized in FigTree ver. 1.4.4 and Adobe Photoshop CC (19.0).

Results

Achnanthidium tineae Tseplik, Kulikovskiy, Kociolek & Maltsev, sp. nov.

Figs 1–3

Holotype. Slide no 04133 in collection of MHA, Main Botanical Garden Russian Academy of Science, Moscow, Russia, represented here by Fig. 1E.

Reference strain. Sample Ind296, isolated in sample I227.

Type locality. Indonesia. Sulawesi Island, Temple Lake, periphyton, 04°06.923'N, 119°58.613'E, 5 m. elev., *leg.* I.I. Ivanov, 22.09.2010.

Description. *LM* (Fig. 1A–R). Frustules rectangular in girdle view, raphe valve very slightly concave. Valves linear-elliptic with gradually narrowing ends. Length 14.7–17.5 μm (16.2 ± 0.9 ; $n=17$), breadth 4.0–5.0 μm (4.5 ± 0.3 ; $n=17$). The raphe valve possesses a straight filiform raphe, which lies in a narrow linear axial area. The central area is represented by a symmetrical butterfly-shaped fascia that reaches the valve margins on both sides. Striae on raphe valve strongly radiate, curved. The rapheless valve possesses a narrow lanceolate axial area. Central area absent, on some valves somewhat shorter striae in the center are present. Striae parallel in the center on the valve, slightly radiate near the valve ends.

SEM, external view (Figs 2A–C, 3A). Central raphe ends are straight and drop-shaped (Fig. 2A, white arrows). Distal raphe ends curve strongly to one side of the valve (Fig. 2A, white arrowheads). Striae on the raphe valve 30–35 in 10 μm (32.5 ± 2.5 in 10 μm ; $n=4$). Areolae elliptical or rounded in shape, approximately 40 in 10 μm . Striae on the rapheless valve 30–33 in 10 μm (31 ± 1.2 in 10 μm ; $n=4$). Areolae small, also rounded or elliptical, approximately 50 in 10 μm . Shorter striae in the center are clearly visible in SEM; this often occurs only on one side of the valve (Fig. 3A, white arrow).

SEM, internal view (Figs 2D, 3B–D). Central raphe ends are simple and straight (Fig. 2D, white arrows). Distal raphe ends terminate in helictoglossae (Fig. 2D, white arrowheads). Shorter striae in the center are clearly visible in SEM, this often occurs only on one side of the valve (Fig. 3B, C, white arrows).

Etymology. Epithet refers to the butterfly-like shape of the fascia on the raphe valve of the new species; *tineae* meaning moth in Latin.

Distribution. As yet known only from type locality.

Molecular data (Fig. 4)

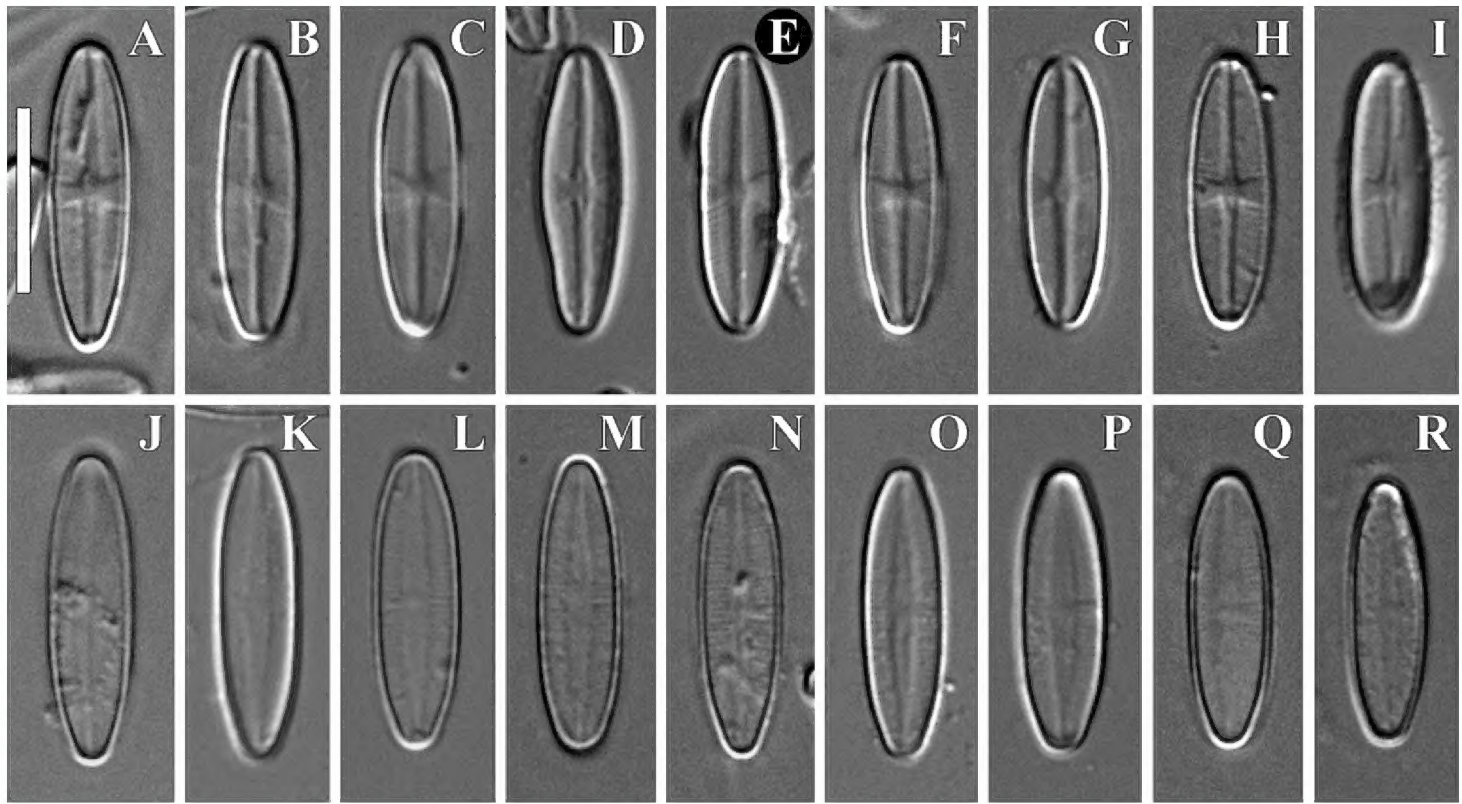


Figure 1. A–R *Achnantheidium tineae* (Tseplik, Kulikovskiy, Kociolek & Maltsev), sp. nov. LM, DIC, size diminution series. Slide no 04133. Holotype (**E**). Scale bar: 10 μ m.

Our new species belongs to the large clade with monoraphid diatoms and sister clade with gomphocymbelloid diatoms. Strain *A. tineae* sp. nov. combined (BI 100; ML 100) with two strains of *A. minutissimum* AW2 and Ashort2 and *A. saprophilum* D06-036. 15 other strains of *A. minutissimum* combined to form a sister branch together with three strains of *A. digitatum* and *A. gladius* Tseplik et al. Other monoraphid taxa from genera *Pauliella*, *Psammothidium*, *Planothidium*, *Cocconeis*, *Lemnicola* and *Gogorovia* spp. formed sister clades to the branch containing these *Achnantheidium* taxa in the molecular tree.

Discussion

In terms of the data from both morphology and molecular sequence data, the new species *A. tineae* sp. nov. belongs to the genus *Achnantheidium*. Morphological features present in *A. tineae* and characteristic for this genus include: linear-elliptical valve shape, sternum that widens near the center of the valve and external distal raphe endings that are curved to one side. The last feature allows us to attribute the new species to the *A. pyrenaicum* species complex.

We compared *A. tineae* sp. nov. with other representatives of the genus *Achnantheidium*. *A. tineae* sp. nov. possesses a rather unusual combination of features for the genus: linear-elliptic valves with parallel sides and narrowed ends and a pronounced butterfly-shaped fascia on the raphe valve. After carrying out the morphological comparison, we identified several species most similar in morphology to *A. tineae* sp. nov.

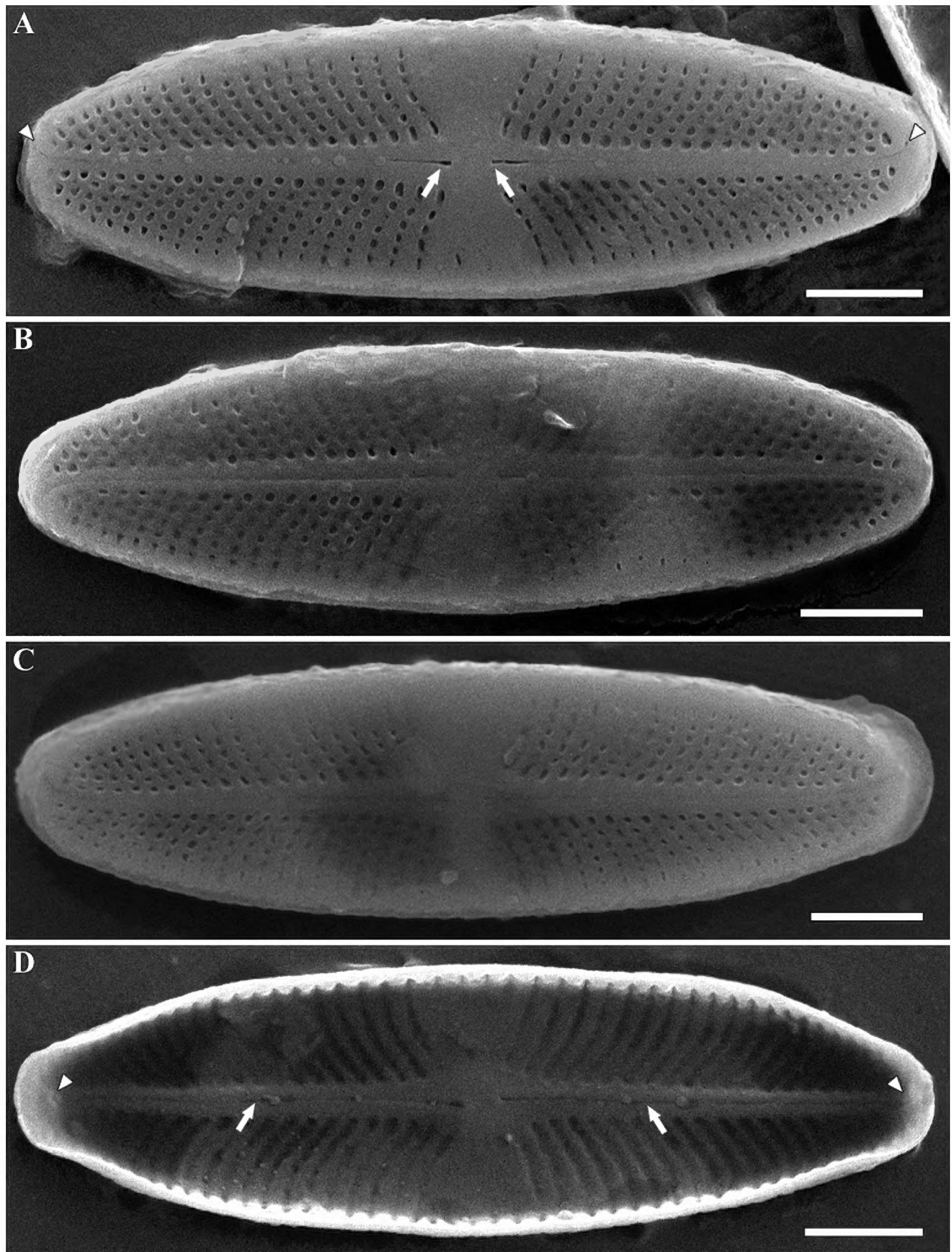


Figure 2. A–D *Achnanthidium tinea* (Tseprik, Kulikovskiy, Kociolek & Maltsev), sp. nov. SEM. Sample no 04133. Raphe valves **A–C** external views **D** internal view **A** white arrows shows the central raphe ends. White arrowheads shows the distal raphe ends **D** white arrows shows the central raphe ends. White arrowheads shows the helictoglossae. Scale bars: 2 μ m.

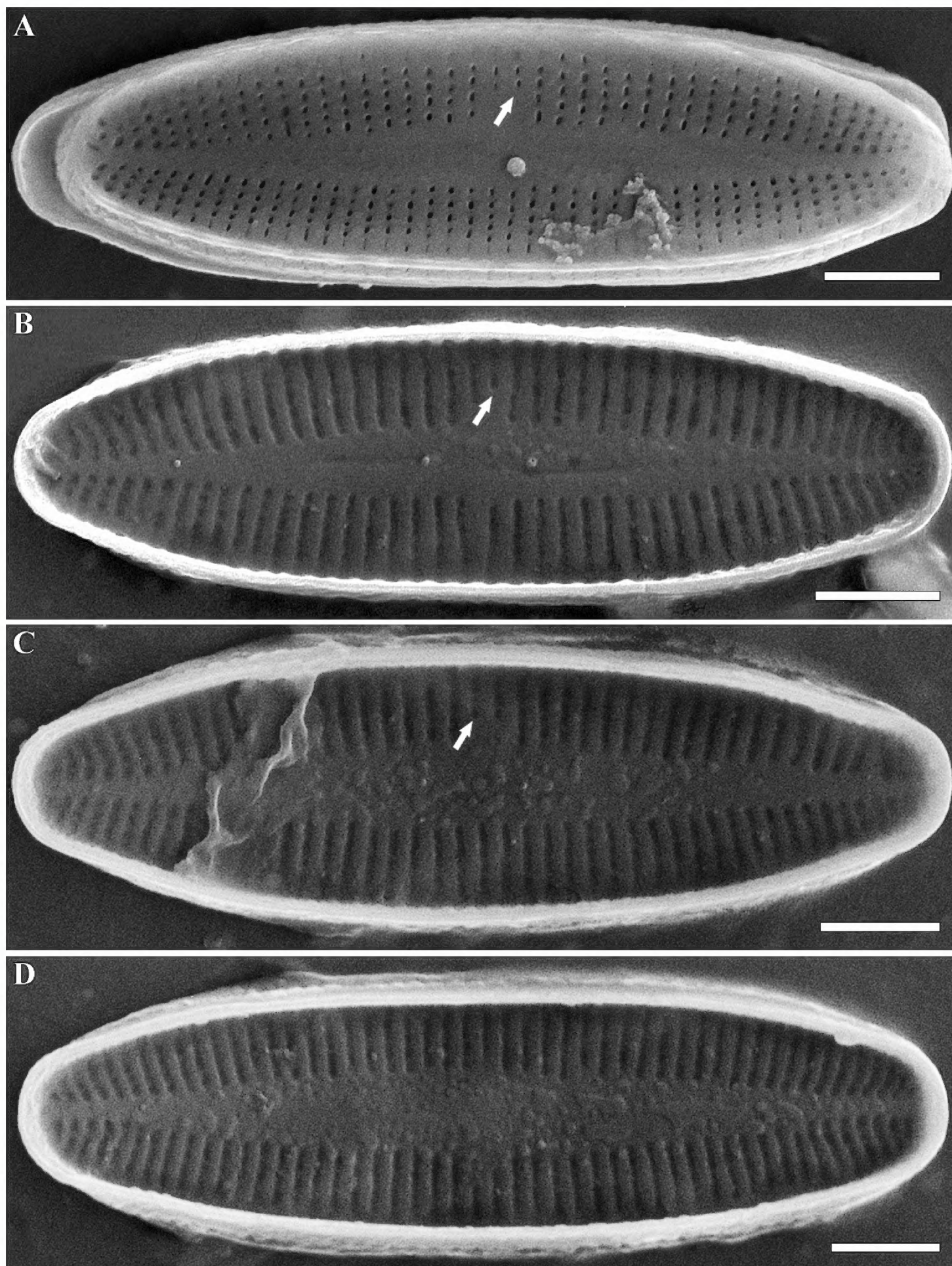


Figure 3. A–D *Achmanthidium tineae* (Tseplik, Kulikovskiy, Kociolek & Maltsev), sp. nov. SEM. Sample no 04133. Rapheless valves. **A** external view **B–D** internal views **A–C** white arrows shows the short striae. Scale bars: 2 μm .



Figure 4. Bayesian tree of *Achnanthidium tinea* (Tseplik, Kulikovskiy, Kociolek & Maltsev), sp. nov. (indicated in bold) constructed from a concatenated alignment of 152 partial 18S rDNA sequences of 404 characters. Values above the horizontal lines are bootstrap support from RAxML analyses (<50 are not shown); values below the horizontal lines and to the right of the slash mark are Bayesian posterior probabilities (<90 are not shown). All sequences have strain numbers (if available). Species of centric diatoms were used as an outgroup. * is 100% statistical support.

In terms of valve shape, the species most similar to the new species is *Achnanthidium deflexum* (Reimer) Kingston (Potapova and Ponader 2004), but it can be quite easily distinguished from *A. tineae* sp. nov. by the absence of the central area and by parallel and more widely spaced striae on the raphe valve (20–22 in 10 μm in *A. deflexum*, 30–35 in 10 μm in *A. tineae* sp. nov.). Another species similar to *A. tineae* sp. nov. in terms of valve shape is *Achnanthidium dolomiticum* Cantonati & Lange-Bertalot (Cantonati and Lange-Bertalot 2006). It differs from the new species by having more widely rounded valve ends and the central area represented by a narrow rectangular fascia. Under the scanning electron microscope, it is also possible to observe straight external distal raphe ends in *A. dolomiticum*, while in *A. tineae* sp. nov. they are curved. *Achnanthidium delmontii* Pérès, Le Cohu & Barthès (Pérès et al. 2012) also resembles *A. tineae* in terms of valve shape and, like *A. tineae* sp. nov., belongs to the *A. pyrenaicum* species complex. But *A. delmontii* has a narrower rectangular fascia and more widely-spaced, weakly radiate striae on both valves (raphe valves: 30–35 in 10 μm in *A. tineae* sp. nov., 20–26 in 10 μm in *A. delmontii*; rapheless valves: 30–33 in 10 μm in *A. tineae* sp. nov., 18–22 in 10 μm in *A. delmontii*). Two other species that somewhat resemble *A. tineae* sp. nov. were studied by Morales et al. (2011), namely *Achnanthidium cadimae* Morales, Fernández & Ector and *Achnanthidium peruvianum* Morales & Ector. *A. cadimae* can be differentiated from our new species by its narrowly elliptic valves that are smaller than *A. tineae* sp. nov. (10–13 μm versus 14.7–17.5 μm), its asymmetrical fascia, and the axial area on its rapheless valve that is very narrow and almost linear versus a somewhat broader lanceolate one in *A. tineae* sp. nov. *A. peruvianum* also has a smaller fascia and a narrower axial area on its rapheless valve than *A. tineae* sp. nov., and its valve ends are more broadly rounded. A final species that is similar in valve shape is *Achnanthes tropica* Hustedt, illustrated with line drawings by Hustedt (1937b, Plate XIII, figs 28–32) and described from Java (Hustedt 1937b, p. 200). This species also has fine striae (reported as “zart” by Hustedt and described as 26–30/10 μm), but coarser than in *Achnanthidium tineae*. While no SEM work has yet been done on Hustedt’s species, the light microscope images of this taxon published by Simonsen (1987, plate 326, figs 20–28) suggest this species might be better placed in the genus *Nupela* Vyverman & Compère.

In general, due to the above-mentioned unusual combination of features possessed by the new species, its similarity with other representatives of the genus is mostly quite superficial, and *A. tineae* sp. nov. is easily distinguishable from other species even in light microscopy.

On the phylogenetic tree, the strain of *A. tineae* sp. nov. forms a separate branch within a clade that includes other species of *Achnanthidium* and other monoraphid diatoms (e.g. *Gogorevia*, *Psammothidium*, *Planothidium*, *Pauliella*, *Cocconeis*, *Lemnicola*) and the Cymbellales. The group was referred to as the Monoplacatae by Mereschowsky (1902) and has been recovered in previous phylogenetic analyses (e.g. Thomas et al. 2016). Within this large group, *A. tineae* belongs to a large clade comprised of strains of several *Achnanthidium* species, including *A. minutissimum*, *Achnanthidium digitatum* Pinseel, Vanormelingen, Hamilton & Van de Vijver, *Achnanthidium saprophilum* (Kobayashi & Mayama) Round & Bukhtiyarova and *Achnanthidium gladius* Tseplik,

Kulikovskiy, Glushchenko & Genkal. As discussed above, morphologically, none of these species is similar to *A. tinea* sp. nov. The clade including this species is sister to another clade that comprises strains of other monoraphid genera, namely *Pauliella* Round & Basson, *Psammothidium* Bukhtiyarova & Round and *Cocconeis* Ehrenberg.

Our understanding of the phylogenetic relationships of the monoraphid diatoms continues to yield fascinating new insights at the levels of genus and species (e.g. Round and Basson 1997; Moser et al. 1998; Witkowski et al. 2000; Krammer and Lange-Bertalot 2004; Riaux-Gobin et al. 2012), and a richer understanding of the evolution of the monoraphid condition (Kociolek et al. 2019). The description of new species within genera such as *Achnanthidium* is quite an important area of research for the taxonomy of this genus, but also shows how the genus might be understood for ecological analyses (Potapova and Hamilton 2007). An integrated molecular and morphological approach to species-level identification and understanding phylogenetic relationships of those taxa will provide a more complete picture of the taxonomy of the genus, allow for the construction of a natural classification, and facilitate further research.

Acknowledgements

The authors are grateful to the staff of the Centre of Electron Microscopy of the Papanin's Institute for Biology of Inland Waters, RAS, for technical assistance. Publication is based on research carried out with financial support by Russian Science Foundation (19–14–00320).

References

- Andersen RA [Ed.] (2005) *Algal Culturing Techniques*. Elsevier Academic Press, Oxford, 596 pp.
- Andreeva SA, Kulikovskiy MS, Maltsev YeI, Podunay YuA, Gusev ES (2016) Molecular genetic investigation of diatom genera *Diadesmis* and *Humidophila* (Bacillariophyceae). *Botanicheskij journal* 101(6): 621–628. <https://doi.org/10.1134/S0006813616060028>
- Cantonati M, Lange-Bertalot H (2006) *Achnanthidium dolomiticum* sp. nov. (Bacillariophyta) from oligotrophic mountain springs and lakes fed by dolomite aquifers (Note). *Journal of Phycology* 42(6): 1184–1188. <https://doi.org/10.1111/j.1529-8817.2006.00281.x>
- Cleve PT (1895) Synopsis of the Naviculoid Diatoms, Part II. *Kongliga Svenska-Vetenskaps Akademiens Handlingar* 27(3): e219.
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9(8): e772. <https://doi.org/10.1038/nmeth.2109>
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7(1): e214. <https://doi.org/10.1186/1471-2148-7-214>
- Glushchenko AM, Genkal SI, Kulikovskiy MS (2016) *Aulacoseira konstantinovii* sp. nov. and *Aulacoseira krylovii* sp. nov. – two new centric diatoms from South-east Asia. *Diatom Research* 31(4): 367–378. <https://doi.org/10.1080/0269249X.2016.1253617>

- Glushchenko AM, Kulikovskiy MS, Kociolek JP (2017) New and interesting species from the genus *Luticola* (Bacillariophyceae) in waterbodies of Southeastern Asia. *Nova Hedwigia. Beiheft* 146: 157–173. <https://doi.org/10.1127/1438-9134/2017/157>
- Glushchenko AM, Kulikovskiy MS, Dorofeyuk NI, Kociolek JP (2018) New species and combinations in the genus *Eunotia* Ehrenberg 1837 (Bacillariophyceae: Eunotiaceae) from waterbodies of Southeastern Asia. *Nova Hedwigia. Beiheft* 147: 69–103. <https://doi.org/10.1127/nova-suppl/2018/009>
- Glushchenko AM, Kociolek JP, Kuznetsova IV, Kezlya EM, Kulikovskiy MS (2019) *Prestauro-neis genkalii* – a new diatom species (Bacillariophyceae: Stauroneidaceae) from Southeast Asia. *Phytotaxa* 414(4): 156–164. <https://doi.org/10.11646/phytotaxa.414.4.1>
- Glushchenko AM, Kociolek JP, Kuznetsova IV, Kulikovskiy MS (2020) Four new species from the diatom (Bacillariophyceae) genus *Adlafia* Moser, Lange-Bertalot & Metzeltin from waterbodies of Vietnam. *PhytoKeys* 162: 13–27. <https://doi.org/10.3897/phytokeys.162.57657>
- Hamsher SE, Graeff CL, Stepanek JG, Kociolek JP (2014) Variation in valve and girdle band morphology in freshwater *Denticula* (Bacillariophyceae) species: Implications for the systematic position of the genus including the description of *Tetralunata* gen. nov. (Epithemiaceae, Rhopalodiales). *Plant Ecology and Evolution* 147: 346–365. <https://doi.org/10.5091/plecevo.2014.990>
- Hlúbíková D, Ector L, Hoffmann L (2011) Examination of the type material of some diatom species related to *Achnantheidium minutissimum* (Kütz.) Czarn. (Bacillariophyceae). *Algalogical Studies* 136/137: 19–43. <https://doi.org/10.1127/1864-1318/2011/0136-0019>
- Hustedt F (1937a) Systematische und ökologische Untersuchungen über die Diatomeen-Flora von Java, Bali und Sumatra nach dem Material der Deutschen Limnologischen Sunda-Expedition. “Tropische Binnengewässer, Band VII. Archiv für Hydrobiologie (Supplement 15): 131–177.
- Hustedt F (1937b) Systematische und ökologische Untersuchungen über die Diatomeen-Flora von Java, Bali und Sumatra nach dem Material der Deutschen Limnologischen Sunda-Expedition. “Tropische Binnengewässer, Band VII. Archiv für Hydrobiologie (Supplement 15): 187–295.
- Hustedt F (1937c) Systematische und ökologische Untersuchungen über die Diatomeen-Flora von Java, Bali und Sumatra nach dem Material der Deutschen Limnologischen Sunda-Expedition. “Tropische Binnengewässer, Band VII. Archiv für Hydrobiologie (Supplement 15): 393–506.
- Hustedt F (1938a) Systematische und ökologische Untersuchungen über die Diatomeen-Flora von Java, Bali und Sumatra nach dem Material der Deutschen Limnologischen Sunda-Expedition. “Tropische Binnengewässer, Band VII. Archiv für Hydrobiologie (Supplement 15): 638–790.
- Hustedt F (1938b) Systematische und ökologische Untersuchungen über die Diatomeen-Flora von Java, Bali und Sumatra nach dem Material der Deutschen Limnologischen Sunda-Expedition. “Tropische Binnengewässer, Band VII. Archiv für Hydrobiologie (Supplement 16): 1–155.
- Hustedt F (1939) Systematische und ökologische Untersuchungen über die Diatomeen-Flora von Java, Bali und Sumatra nach dem Material der Deutschen Limnologischen Sunda-Expedition. “Tropische Binnengewässer, Band VII. Archiv für Hydrobiologie (Supplement 16): 274–394.

- Hustedt F (1942) Süßwasser-Diatomeen des indomalayischen Archipels und der Hawaii-In-slen. Internationale Revue der Gesamten Hydrobiologie und Hydrographie 42: 1–252. <https://doi.org/10.1002/iroh.19420420102>
- Jahn R, Abarca N, Gemeinholzer B, Mora D, Skibbe O, Kulikovskiy M, Gusev E, Kusber W-H, Zimmermann J (2017) *Planothidium lanceolatum* and *Planothidium frequentissimum* reinvestigated with molecular methods and morphology: Four new species and the taxonomic importance of the sinus and cavum. Diatom Research 32(1): 75–107. <https://doi.org/10.1080/0269249X.2017.1312548>
- Kapustin DA, Kulikovskiy MS, Kociolek JP (2017) *Celebesia* sp. nov., a new cymbelloid diatom genus from the ancient lake Matano (Sulawesi Island, Indonesia). Nova Hedwigia. Beiheft 146: 147–155. <https://doi.org/10.1127/1438-9134/2017/147>
- Kapustin DA, Kociolek JP, Glushchenko AM, Kulikovskiy MS (2019) Four new species of *Cymbella* (Bacillariophyta) from the ancient Malili lakes (Sulawesi Island, Indonesia). Botanicheskij journal 104(5): 766–780. <https://doi.org/10.1134/S0006813619050065>
- Kapustin DA, Kociolek JP, Glushchenko AM, Kulikovskiy MS (2020) A rediscovery of *Cymbella mirabilis* Hustedt, a rare endemic diatom, and description of *Alveocymba* gen. nov. Diatom Research 35(3): 281–287. <https://doi.org/10.1080/0269249X.2020.1772888>
- Karthick B, Taylor JC, Hamilton PB (2017) Two new species of *Achnanthidium* Kützing (Bacillariophyceae) from Kolli Hills, Eastern Ghats, India. Fottea 17(1): 65–77. <https://doi.org/10.5507/fot.2016.020>
- Katoh K, Toh H (2010) Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics (Oxford, England) 26(15): 1899–1900. <https://doi.org/10.1093/bioinformatics/btq224>
- Kezlya EM, Glushchenko AM, Maltsev YeI, Gusev ES, Genkal SI, Kuznetsov AN, Kociolek JP, Kulikovskiy MS (2020) *Placoneis cattiensis* sp. nov. a new, diatom (Bacillariophyceae: Cymbellales) soil species from Cát Tiên National Park (Vietnam). Phytotaxa 460(4): 237–248. <https://doi.org/10.11646/phytotaxa.460.4.1>
- Kobayashi H (1997) Comparative studies among four linear-lanceolate *Achnanthidium* species (Bacillariophyceae) with curved terminal raphe endings. Nova Hedwigia 65(1–4): 147–164. <https://doi.org/10.1127/nova.hedwigia/65/1997/147>
- Kociolek JP, Kapustin DA, Kulikovskiy MS (2018) A new, large species of *Gomphonema* Ehrenberg from ancient Lake Matano, Indonesia. Diatom Research 33(2): 241–250. <https://doi.org/10.1080/0269249X.2018.1513868>
- Kociolek JP, Williams DM, Stepanek J, Liu Q, Liu Y, You Q-M, Karthick B, Kulikovskiy MS (2019) Rampant homoplasy and adaptive radiation in pennate diatoms. Plant Ecology and Evolution 152(2): 131–141. <https://doi.org/10.5091/plecevo.2019.1612>
- Kociolek JP, Balasubramanian K, Blanco S, Coste M, Ector L, Liu Y, Kulikovskiy MS, Lundholm N, Ludwig T, Potapova M, Rimet F, Sabbe K, Sala S, Sar E, Taylor J, Van de Vijver B, Wetzel CE, Williams DM, Witkowski A, Witkowski J (2020) DiatomBase. <https://www.diatombase.org> [searched on 30 April 2020]
- Krahn KJ, Wetzel CE, Ector L, Schwalb A (2018) *Achnanthidium neotropicum* sp. nov., a new freshwater diatom from Lake Apastepeque in El Salvador (Central America). Phytotaxa 382(1): 89–101. <https://doi.org/10.11646/phytotaxa.382.1.4>

- Krammer K, Lange-Bertalot H (2004) Bacillariophyceae 4. Teil: Achnanthaceae, Kritische Ergänzungen zu *Navicula* (Lineolatae), *Gomphonema* Gesamtliteraturverzeichnis Teil 1–4 (second revised edition) (With “Ergänzungen und Revisionen” by H. Lange Bertalot). In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (Eds) Süßwasserflora von Mitteleuropa (Vol. 2(4)). Spektrum Akademischer Verlag, Heidelberg, 468 pp.
- Kulikovskiy MS, Lange-Bertalot H, Witkowski A, Khursevich GK (2011) *Achnanthidium sibiricum* (Bacillariophyceae), a new species from bottom sediments in Lake Baikal. *Algological Studies* 136/137: 77–87. <https://doi.org/10.1127/1864-1318/2011/0136-0077>
- Kulikovskiy MS, Lange-Bertalot H, Metzeltin D, Witkowski A (2012) Lake Baikal: Hotspot of endemic diatoms I. *Iconographia Diatomologica* 23: 7–607.
- Kulikovskiy MS, Lange-Bertalot H, Witkowski A (2013) *Gliwiczia* gen. nov. a new monoraphid diatom genus from Lake Baikal with a description of four species new for science. *Phytotaxa* 109(1): 1–16. <https://doi.org/10.11646/phytotaxa.109.1.1>
- Kulikovskiy MS, Gusev ES, Andreeva SA, Annenkova NV (2014) Phylogenetic position of the diatom genus *Geissleria* Lange-Bertalot & Metzeltin and description of two new species from Siberian mountain lakes. *Phytotaxa* 177(5): 249–260. <https://doi.org/10.11646/phytotaxa.177.5.1>
- Kulikovskiy MS, Lange-Bertalot H, Kuznetsova IV (2015) Lake Baikal: Hotspot of endemic diatoms II. *Iconographia Diatomologica* 26: 1–657.
- Kulikovskiy MS, Andreeva SA, Gusev ES, Kuznestova IV, Annenkova NV (2016a) Molecular phylogeny of monoraphid diatoms and raphe significance in evolution and taxonomy. *The Biological Bulletin* 43(5): 398–407. <https://doi.org/10.1134/S1062359016050046>
- Kulikovskiy MS, Lange-Bertalot H, Annenkova NV, Gusev ES, Kociolek JP (2016b) Morphological and molecular evidence support description of two new diatom species from the genus *Ulnaria* in Lake Baikal. *Fottea, Olomouc* 16(1): 34–42. <https://doi.org/10.5507/fot.2015.011>
- Kulikovskiy MS, Lange-Bertalot H, Kuznetsova IV (2016c) *Cocconeis nanoburyatica* sp. nov. – a new monoraphid diatom species from Lake Baikal. *Inland Water Biology* 9(2): 112–115. <https://doi.org/10.1134/S1995082916020103>
- Kulikovskiy MS, Glushchenko AM, Kuznetsova IV, Kociolek JP (2018) Description of the new freshwater diatom genus *Okhaphkinia* gen. nov. from Laos (Southeast Asia), with notes on family Sellaphoraceae Mereschkowsky 1902. *Fottea, Olomouc* 18(1): 120–129. <https://doi.org/10.5507/fot.2017.021>
- Kulikovskiy MS, Kapustin DA, Glushchenko AM, Sidelev S, Maltsev YeI, Gusev ES, Kezlya EM, Shkurina N, Kuznetsova IV, Kociolek JP (2020a) Morphological and molecular investigation of *Gomphonema longissimum* and related taxa from Malili lakes (Indonesia) with comments on diatom evolution in ancient lakes. *European Journal of Phycology* 55(2): 147–161. <https://doi.org/10.1080/09670262.2019.1664771>
- Kulikovskiy MS, Maltsev YeI, Andreeva SA, Glushchenko AM, Gusev ES, Podunay YuA, Ludwig TV, Tusset E, Kociolek JP (2019) Description of a new diatom genus *Dorofeyukea* gen. nov. with remarks on phylogeny of the family Stauroneidaceae. *Journal of Phycology* 55(1): 173–185. <https://doi.org/10.1111/jpy.12810>

- Kulikovskiy MS, Glushchenko AM, Genkal SI, Kuznetsova IV, Kociolek JP (2020b) *Platebailalia* – a new monoraphid diatom genus from ancient Lake Baikal with comments on the genus *Platessa*. Fottea, Olomouc 20(1): 58–67. <https://doi.org/10.5507/fot.2019.014>
- Kulikovskiy MS, Maltsev YeI, Glushchenko AM, Kuznetsova IV, Kapustin DA, Lange-Bertalot H, Genkal SI, Kociolek JP (2020c) *Gogorevia*, a new monoraphid diatom genus for *Achnanthes exigua* and allied taxa (Achnanthidiaceae) described on the basis of an integrated molecular and morphological approach. Journal of Phycology 56(6): 1601–1613. <https://doi.org/10.1111/jpy.13064>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33(7): 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Kützing FT (1844) Die kieselschaligen Bacillarien oder Diatomeen. Nordhausen: zu finden bei W. Köhne, 152 pp. <https://doi.org/10.5962/bhl.title.64360>
- Liu Y, Kociolek JP, Glushchenko AM, Kulikovskiy MS, Fan Y (2018) A new genus of Eunotiales (Bacillariophyta, Bacillariophyceae: Peroniaceae), *Sinoperonia*, from Southeast Asia, exhibiting remarkable phenotypic plasticity, and evidence for another lineage of monoraphid diatoms. Phycologia 57(2): 147–158. <https://doi.org/10.2216/17-21.1>
- Maltsev YeI, Kulikovskiy MS (2017) Morphological and genetic variability of *Hantzschia amphioxys* (Bacillariophyceae) in terrestrial and aquatic habitats. Botanicheskij journal 102(1): 17–35. <https://doi.org/10.1134/S0006813617010021>
- Maltsev YeI, Andreeva SA, Kulikovskiy MS, Podunay YuA, Kociolek JP (2018) Molecular phylogeny of the diatom genus *Envekadea* (Bacillariophyceae, Naviculales). Nova Hedwigia. Beiheft 146: 241–252. <https://doi.org/10.1127/1438-9134/2017/241>
- Maltsev YeI, Andreeva SA, Podunay YuA, Kulikovskiy MS (2019) Description of *Aneumastus mongolotusculus* sp. nov. (Bacillariophyceae, Mastogloiales) from Lake Hovsgol (Mongolia) on the basis of molecular and morphological investigations. Nova Hedwigia. Beiheft 148: 21–33. <https://doi.org/10.1127/nova-suppl/2019/035>
- Mereschkowsky C (1902) Les types de l'endochrome chez les Diatomées. Scripta Botanica Horti Universitatis (Imperialis) 21: 1–193.
- Morales EA, Ector L, Fernández E, Novais MH, Hlúbikova D, Hamilton PB, Blanco S, Vis ML, Kociolek JP (2011) The genus *Achnanthidium* Kütz. (Achnanthales, Bacillariophyceae) in Bolivian streams: A report of taxa found in recent investigations. Algological Studies 136/137: 89–130. <https://doi.org/10.1127/1864-1318/2011/0136-0089>
- Moser G, Lange-Bertalot H, Metzeltin D (1998) Insel der Endemiten Geobotanisches Phänomen Neukaledonien (Island of endemics New Caledonia – a geobotanical phenomenon). Bibliotheca Diatomologica 38: 1–464.
- Novais MH, Hlúbiková D, Morais M, Hoffmann L, Ector L (2011) Morphology and ecology of *Achnanthidium caravelense* (Bacillariophyceae), a new species from Portuguese rivers. Algological Studies 136/137: 131–150. <https://doi.org/10.1127/1864-1318/2011/0136-0131>
- Pérès F, Barthès A, Ponton E, Coste M, Ten-Hague L, Le-Cohu R (2012) *Achnanthidium delmontii* sp. nov., a new species from French rivers. Fottea 12(2): 189–198. <https://doi.org/10.5507/fot.2012.014>

- Ponader KC, Potapova MG (2007) Diatoms from the genus *Achnantheidium* in flowing waters of the Appalachian Mountains (North America): Ecology, distribution and taxonomic notes. *Limnologia* 37(3): 227–241. <https://doi.org/10.1016/j.limno.2007.01.004>
- Potapova MG, Hamilton PB (2007) Morphological and ecological variation within the *Achnantheidium minutissimum* (Bacillariophyceae) species complex. *Journal of Phycology* 43(3): 561–575. <https://doi.org/10.1111/j.1529-8817.2007.00332.x>
- Potapova MG, Ponader KC (2004) Two common North American diatoms, *Achnantheidium rivulare* sp. nov. and *A. deflexum* (Reimer) Kingston: Morphology, ecology and comparison with related species. *Diatom Research* 19(1): 33–57. <https://doi.org/10.1080/0269249X.2004.9705606>
- Riaux-Gobin C, Witkowski A, Ruppel M (2012) *Scalariella* a new genus of monoraphid diatom (Bacillariophyta) with a bipolar distribution. *Fottea* 12(1): 13–25. <https://doi.org/10.5507/fot.2012.002>
- Rimet F, Couté A, Piuz A, Berthon V, Druart J-C (2010) *Achnantheidium druartii* sp. nov. (Achnanthes, Bacillariophyta), a new species invading European rivers. *Vie et Milieu* 60(3): 185–195.
- Round FE, Basson PW (1997) A new monoraphid diatom genus (*Pogoneis*) from Bahrain and the transfer of previously described species *A. hungarica* and *A. taeniata* to new genera. *Diatom Research* 12(1): 71–81. <https://doi.org/10.1080/0269249X.1997.9705403>
- Round FE, Bukhtiyarova LN (1996) Four new genera based on *Achnanthes* (*Achnantheidium*) together with a re-definition of *Achnantheidium*. *Diatom Research* 11(2): 345–361. <https://doi.org/10.1080/0269249X.1996.9705389>
- Round FE, Crawford RM, Mann DG (1990) *The Diatoms. Biology and Morphology of the Genera*. Cambridge University Press, Cambridge, 747 pp.
- Rybak M, Solak CN, Noga T, Glushchenko AM, Williams DM, Kulikovskiy MS (2019) *Nupela brevistriata* sp. nov. – a new, terrestrial diatom species from Southeast Asia. *Diatom Research* 34(4): 251–258. <https://doi.org/10.1080/0269249X.2019.1698467>
- Simonsen R (1987) *Atlas and catalogue of the diatom types of Friedrich Hustedt*. Cramer bei Borntraeger, Berlin und Stuttgart. [Vol. 1 Catalogue: x, 525; Vol. 2 Atlas: pls 1–395; Vol. 3 Atlas, pls 396–772]
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* 57(5): 758–771. <https://doi.org/10.1080/10635150802429642>
- Thomas E, Stepanek J, Kociolek JP (2016) Historical and current perspectives on the systematics of the ‘enigmatic’ diatom genus *Rhoicosphenia* (Bacillariophyta), with single and multi-molecular marker and morphological analyses and discussion on the monophyly of ‘monoraphid’ diatoms. *PLoS ONE* 11(4): e0152797. <https://doi.org/10.1371/journal.pone.0152797>
- Tseplik ND, Maltsev YeI, Glushchenko AM, Kuznetsova IV, Genkal SI, Kulikovskiy MS (2020) (in press) *Achnantheidium gladius* sp. nov. (Bacillariophyceae) – a new monoraphid diatom species from Indonesia. *Inland Water Biology*.
- Van de Vijver B, Ector L, Beltrami ME, de Haan M, Falasco E, Hlúbíková D, Jarlman A, Kelly M, Novais MH, Wojtal AZ (2011) A critical analysis of the type material of *Achnantheidium lineare* W. Sm. (Bacillariophyceae). *Algological Studies* 136/137: 167–191. <https://doi.org/10.1127/1864-1318/2011/0136-0167>

- Witkowski A, Lange-Bertalot H, Metzeltin D (2000) Diatom Flora of Marine Coasts I. Iconographia Diatomologica 7: 1–925.
- Yu P, You Q, Kociolek JP, Wang Q (2019) Three new freshwater species of the genus *Achnanthidium* (Bacillariophyta, Achnanthidiaceae) from Taiping Lake, China. Fottea 19(1): 33–49. <https://doi.org/10.5507/fot.2018.015>
- Zimmermann J, Jahn R, Gemeinholzer B (2011) Barcoding diatoms: Evaluation of the V4 sub-region on the 18S rRNA gene, including new primers and protocols. Organisms, Diversity & Evolution 11(3): 173–192. <https://doi.org/10.1007/s13127-011-0050-6>

Supplementary material I

Taxa and DNA sequence data used in phylogenetic analysis

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Data type: species data

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Link: <https://doi.org/10.3897/phytokeys.174.60337.suppl1>